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OBSERVATIONS ON EGG-LAYING IN *ILYBIUS FULIGINOSUS* FABRICIUS AND *I. ATER* DEGEER (COLEOPTERA : DYTISCIDAE), WITH AN ACCOUNT OF THE FEMALE GENITALIA.

By Dorothy J. Jackson.

(*North Cliff, St. Andrews, Fife*)

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With 9 Text-figures

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I. Introduction and Acknowledgments

It is well known that the females of *Ilybius* are provided with toothed ovipositor blades by means of which they insert their eggs in the tissues of aquatic plants. Last summer I was able to watch the oviposition of *I. fuliginosus* and *I. ater* on various occasions, and, as the process of laying does not appear to have been described before, the following observations may be of interest. I am most grateful to Mr. J. Balfour-Browne of the British Museum (Natural History) for reading over and commenting on the typescript of this paper.

I have had beetles of these two species laying in my laboratory for the last few summers in order to use their eggs for rearing the summer generations of the Mymarid, *Caraphractus cinctus* Walker. I have also observed the eggs of these beetles *in situ* on various aquatic plants in ponds. The eggs may be found beneath scars in the plant tissue, and, by keeping them until they hatch, it is possible to identify the larvae as those of *Ilybius*, since they differ in various particulars from the newly hatched larvae of *Agabus* (Bertrand, 1928). The oviposition by *Ilybius* within plant tissue is very different from that of some species of *Agabus* already recorded (Jackson, 1958*a*), and the laying apparatus is greatly modified ; an account is therefore given of the principal parts of the female genitalia in *Ilybius*.

II. *Ilybius fuliginosus* Fabricius

1. *Seasonal Appearance and Life Cycle*

This species is common in the summer months in ponds, streams and running ditches. It becomes scarce in water in the late autumn but reappears in spring. The latest date I have netted a specimen is 10th October and the earliest 30th April. Wesenberg-Lund (1912 : 60) states that *Ilybius* occurs in winter in earth holes near the water margin or under stones. I have taken specimens of *I. fuliginosus* in moss on a bank overhanging a loch in the island of Raasay as early as the end of September. Balfour-Browne (1950 : 166–167) states that the imago disappears into hibernation in September and October and reappears towards the end of April. I have taken soft specimens in Fife only in June and July, the same months as recorded by Balfour-Browne (1950 : 272). From an egg laid in early August a larva was successfully reared. It was kept in a tumbler in an unheated room and it fed throughout the autumn and winter on *Tubifex* worms. It ceased feeding in the middle of February and so was removed to a small cup of water embedded in earth. It disappeared into the soil, and on 10th May a lively female with soft elytra had emerged. Out of doors its development would probably have taken longer. This specimen, and other soft imagines, had the ovaries minute and quite immature, so, since emergence takes place in summer and oviposition also occurs in summer, it seems likely that the beetles which lay in the early part of the summer will have hibernated as imagines. Moreover dissection has shown that some of the females with hardened cuticle collected from June to August have the ovaries immature.

2. *Period of Oviposition*

Eggs have been laid in captivity from early June till the beginning of September but the principal laying period in Fife is from the end of June until early August. Females collected in the end of April did not lay till early June.

3. *The Female Genitalia*

Introductory.—Preliminary examination of the female genitalia in *Ilybius* led me to suspect that there were two genital openings, such as Heberdey (1931) described in *Hydroporus ferrugineus* Steph., the upper leading into the bursa copulatrix and the lower into the vagina. Heberdey states that this condition is unique among beetles, and as far as I am aware no other instances have since been recorded in the Coleoptera. In order to ascertain whether two genital orifices occurred in *Ilybius*, dissections of the reproductive organs of both *I. fuliginosus* and *I. ater* have been made. The beetles were either newly killed and dissected in water, or placed first for a few hours in the fixative described by Burke (1959). This fixative has proved very useful in clarifying the dissection and also in rendering the reproductive organs less liable to break. It consists of 5 ml. of 40 per cent. formaldehyde, 2·5 ml. of glacial acetic acid and 20 grms. of chloral hydrate, mixed together and diluted to 100 ml. with distilled water. The beetles were killed with chloroform vapour, the head and prothorax cut off, the elytra removed and the abdominal tergites slit down the centre. The reproductive organs were stained in acid fuchsin, aceto-carmine or light green and mounted in euparal or balsam. Observations were also made on the genital openings in whole specimens in which the laying apparatus was normally extended, while very useful data on the anatomy were afforded by compressing the abdomen of a newly killed female when the membranous parts at the apex of the abdomen, including the genital openings, became protruded. By immersion in alcohol these parts could be preserved in their distended condition and made into permanent mounts. The various sclerites of the laying apparatus were examined *in situ* and also after dissection, when cleared and mounted.

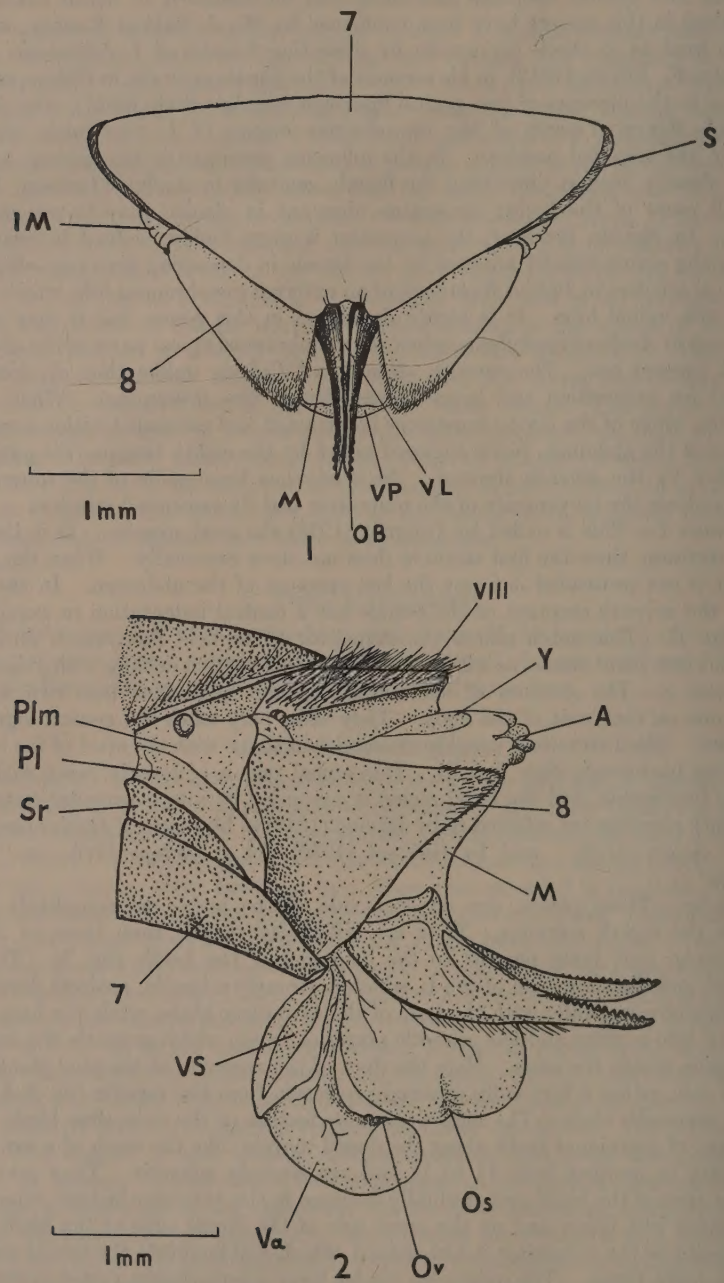
As a result of these investigations conclusive evidence has been obtained that there are two genital openings and these will be described in detail below. My conclusions in this respect have been confirmed by Mr. J. Balfour-Browne, who has been so kind as to check my results by dissecting females of *I. fuliginosus* and *I. fenestratus* F. Böving (1913), in his account of the female genitalia in *Ilybius*, made no reference to the presence of two genital openings, nor does Stein (1847), who gives an admirable figure of parts of the reproductive organs of *I. fenestratus*, omitting, however, the terminal portions. In the following account the terminology adopted is that already used in describing the female genitalia in *Agabus* (Jackson, 1958*a*), since all parts of the laying apparatus observed in *Agabus* may be recognized in *Ilybius*. In *Ilybius*, however, the ovipositor is more highly evolved in relation to the piercing action usually adopted by the female in depositing her eggs. Moreover the vulval sclerites in *Ilybius* form part of an external membranous lobe which I have termed the vulval lobe. It is highly developed in this genus, but it may also be recognized in *Agabus bipustulatus*, when the membranous apical parts of the abdomen are not pressed out. The seventh sternum of *Ilybius*, unlike that of *Agabus*, is modified for oviposition and hence is included in this description. When not in action the whole of the laying apparatus is retracted and concealed within a recess in the apex of the abdomen, being enclosed above by the eighth tergum (the pygidium) and below by the seventh sternum. No study has been made of the musculature which controls the movements of the ovipositor and its associated sclerites.

Sternum 7.—This is called by Guignot (1933) the anal sternite. It is the sixth visible sternum, since the first sternum does not show externally. When the genital segment is not protruded it forms the last sternum of the abdomen. In the genus *Ilybius* the seventh sternum of the female has a central indentation or notch at its apex (fig. 1). This notch allows the ovipositor to be held downwards during the initial cut into plant tissue, as will be described in the section dealing with oviposition.

Sternite 8.—The sternum of the eighth segment is divided into two separate plates, one on each side of the body. They show only when the genital segment is protruded. Each sternite is roughly triangular in shape with the apex of the triangle projecting backwards (figs. 2 & 5). This apical region is densely beset with setae directed backwards, and the basal region is concealed by inter-segmental membrane. The eighth sternites are referred to by Böving (1913) in his figure of *Ilybius fenestratus* F. as " cloaca valves ", and by Guignot (1933), for *I. guttiger* Gyll., as " valves génitales ".

Valvifer.—These plates, one on each side of the body, are completely hidden beneath the eighth sternites. They are differently shaped from those of *Agabus*, their greater part lying parallel to the long axis of the beetle (fig. 3). They are L-shaped and the short arm of the L, which is strongly sclerotic, projects downwards and articulates ventrally with the base of the ovipositor blade, while the long arm is produced into a wider and less sclerotic posterior region which supports the membranous region beside the anus. Here the duct of the reservoir of the anal gland opens on each side within a forwardly directed projection from the valvifer (fig. 3 *X*).

The ovipositor blade.—The most noticeable feature of the ovipositor blade (fig. 3) is the row of prominent teeth along the dorsal margin, like the teeth of a saw. The teeth vary in number from 11 to 14 and are strongly sclerotic. They are largest near the apex of the blade and gradually decrease in size towards the base, where they are situated just below and on the outer side of the dorsal edge of the blade. The actual point of the ovipositor is awl-shaped, which will facilitate the initial puncture into the plant tissue. The ovipositor blades have a remarkably varied armature of bristles. The narrow piercing portion of the blade is beset with very short scattered setae which arise from peg-like bases and face away from the point of the blade. They are probably sensory bristles and their position is such as not to hinder the penetration of the blade. Along the middle lower margin of the ovipositor blade



FIGS. 1–2.—*Ilybius fuliginosus* F.: (1) ventral view of apex of abdomen of female showing median apical emargination of seventh sternum (the genital segment is partially everted); (2) lateral view of apex of abdomen of female showing membranous parts fully extruded by pressure on the abdomen.

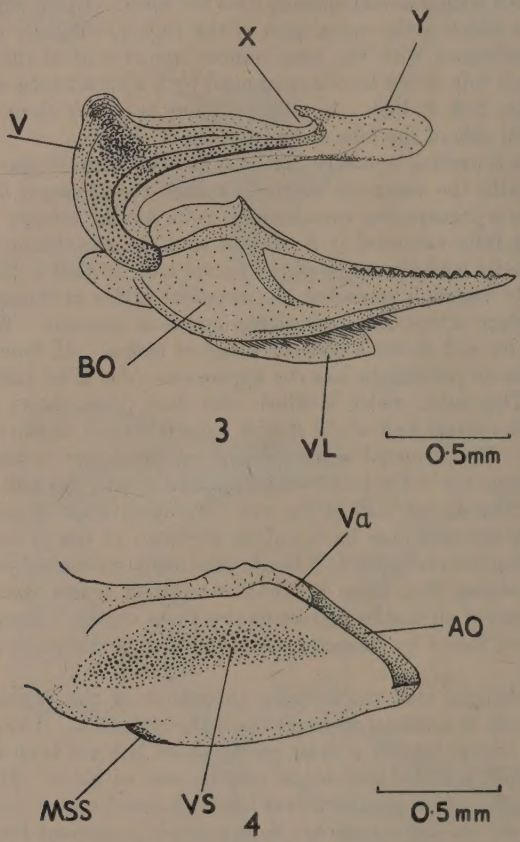
there are from 40 to 50 bristles, mostly very long, and all facing downwards and towards the apex of the blade. It seems probable that they will help to keep the egg in the right position as it is inserted. The inner surface of each ovipositor blade in the middle region is fleshy, and thickly clothed with flat, triangular, scale-like setae which taper to a point, and all are directed towards the apex of the blade with a tilt ventralwards. There is also a scattered sprinkling of long bristles near the base of each blade on the outer side.

The vulval lobe and the genital openings.—When the laying apparatus is not in use, the vulval lobe, a projecting lobe tapering to a blunt point, lies between and partially below the basal portions of the ovipositor blades. It has the appearance of a trough-like sheath with a dorsal opening near the apex. Lying within it is a folded membranous tube which is the apical part of the vagina. Basally the cuticle of the vulval lobe is continuous with the membranous apical wall of the abdomen. The central area of each side of the lobe is reinforced by a long sclerotic strip which is the vulval sclerite (figs. 2 & 4, *VS*). In addition there is a very short median sclerotic area on the ventral side of the lobe at its base (fig. 4, *MSS*).

When pressure is exerted upon the abdomen (and sometimes also when the female dies a natural death) the vagina is everted through the apparent dorsal opening of the vulval lobe as a transparent membranous tube having its own tracheal supply. When the tube is fully extruded it is evident that the membrane of the vagina is continuous externally with the edges of the vulval lobe, so that a double walled tube results. When the vagina is retracted, the apparent orifice at the apex of the vulval lobe marks the place where the vaginal membrane is inflexed. When the tube is partially everted its end shows a distinct inverted orifice. If, however, the vagina is forced out as far as possible, it has the appearance of a blind tube curving dorsalwards (fig. 2). This tube, which is filled with fluid, then shows a rounded end; this is because the ventral wall of the double tube is dilated at the apex by pressure of the body fluid. The inverted orifice remains on the dorsal surface, looking like a fold in the membrane but a fine bristle can be passed within this fold without piercing the membrane. The dorsal wall of the everted vagina bears a group of V-shaped spicules. Similar spicules may be found on the wall of the posterior part of the vagina by dissecting the abdomen of a beetle that has not been subjected to pressure. Stein (1847 : 44) states that these bristle-like teeth or scales are characteristic of the common oviduct of all beetles and he figures them on the " Eiergang " of *Ilybius fenestratus*. I have found by dissection that the vagina continues directly into the vulval lobe.

It might be thought that the extreme protrusion of the vagina here described is entirely the result of artificial pressure upon the abdomen. This, however, is not the case. If the vulval lobe of a dead beetle which has not been subjected to any pressure is examined, a folded membrane may be seen within it. If a fine dissecting needle is put into the orifice on the vulval lobe this membrane is easily brought out, and, though creased, its dimensions are large enough to account for the greater part of the everted tube shown in figure 2. This terminal part of the vagina is undoubtedly capable of extension during laying, when it will be everted through the apex of the vulval lobe as the egg is deposited. The extension of the tube will be caused by pressure of the body fluid during laying. The vulval lobe thus affords a protective outer sheath for the vagina, and will facilitate the deposition of the egg in the slit cut by the ovipositor blades in the plant tissue. Böving (1913), describing the female genitalia of *Ilybius*, attributes a cutting action to the lower margin of the vulval sclerite (which he terms the vulvar sclerite), but as these sclerites do not reach the ventral margin of the lobe and have thus no cutting edge, I consider such an interpretation to be erroneous. All the cutting is done by the ovipositor blades, as I have witnessed.

The opening of the bursa copulatrix lies between the bases of the ovipositor blades and so is dorsal to the vulval opening and ventral to the anus. The opening is quite evident when a freshly killed beetle, in which the genital parts are normally extended, is examined and dissection shows that it is continuous with the relatively large bursa copulatrix. If the abdomen is pressed, the membrane around the anus protrudes, the vagina is everted at the vulval lobe and a large membranous bulge with an inverted orifice protrudes above the vagina. This is the opening of the bursa copulatrix. From it, both in *I. fuliginosus* and in *I. ater*, I have frequently found projecting the tubular prolongation of a spermatophore, and, on dissection of the bursa, the remainder of the spermatophore was obtained.



FIGS. 3–4—(3) *Ilybius fuliginosus* F.: parts of laying apparatus dissected out of a female. (4) *Ilybius ater* Degeer: vulval lobe in lateral view.

There are thus in *Ilybius* two genital openings, and the names I have used for them are those given by Wigglesworth (1950) in his figure of the female reproductive system in Lepidoptera (*after* Eidmann, 1929–1931). As in *Hydroporus*, studied by Heberdey, the upper opening in *Ilybius* is the ostium of the bursa (the copulatory opening) and the lower is the ovipore by which the eggs are laid. In the Lepidoptera, with two genital openings, the relative position of the two openings is reversed. According to Eidmann, the upper opening, which he names the oviporus, is situated on the fused ninth and tenth segments beside the anal opening, while the ostium bursae opens ventrally on the sternal region of the eighth segment or in the inter-

segmental cuticle between the seventh and eighth segments. A similar account of the genital openings in Lepidoptera is given by Weidner (1935).

In *Ilybius*, dissection has failed to show any communication between the vagina and the cavity of the bursa copulatrix. In both *I. fuliginosus* and *I. ater* the short and membranous common oviduct passes into a ventral cavity in the bursa, which appears to be completely cut off by membrane from the main cavity of the bursa.

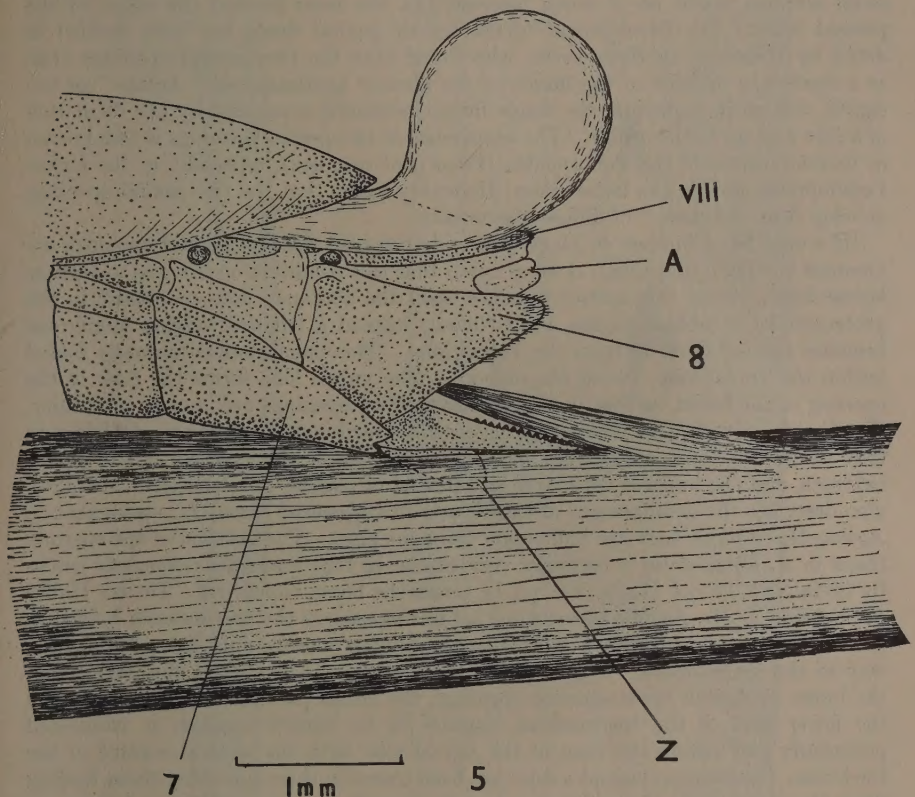


FIG. 5.—*Ilybius fuliginosus* F.: Terminal segments of female during sawing of leaf of *Juncus articulatus*, showing " tongue " of leaf already cut (the female is under water and shows a large air bubble).

Heberdey, in *Hydroporus*, finds that there is a complete separation of the vagina from the bursa copulatrix, and in his figure of a longitudinal section of a female pupa he shows a long duct connecting the receptaculum seminis with the vagina. In *Ilybius fuliginosus* it can be clearly seen that the circular muscles around the ventral wall of the bursa enclose the vagina ; this might give rise to the idea that the common oviduct discharged into a genital chamber, but dissection of the bursa reveals the ventral partition. In *I. fenestratus*, Mr. Balfour-Browne informs me he finds that the vagina lies closely adpressed to the ventral wall of the bursa, but that it is definitely not enclosed by the circular musculature of the bursa, so there may be variation in this character between different species.

The spermatheca in *Ilybius* is a tubular gland opening out of the bursa copulatrix. A second tubular gland arises from the bursa, an accessory gland, the function of which is unknown. Curiously enough in *I. fuliginosus* it is the terminal gland of the bursa

which is the spermatheca (as is shown also by Stein for *I. fenestratus*), but in *I. ater* I have found the spermatozoa in the posterior gland situated on the ventral surface of the bursa. In both species a fertilization canal leads from the spermatheca down the ventral partition of the bursa into the vagina, and I have traced it almost to the vulval lobe.

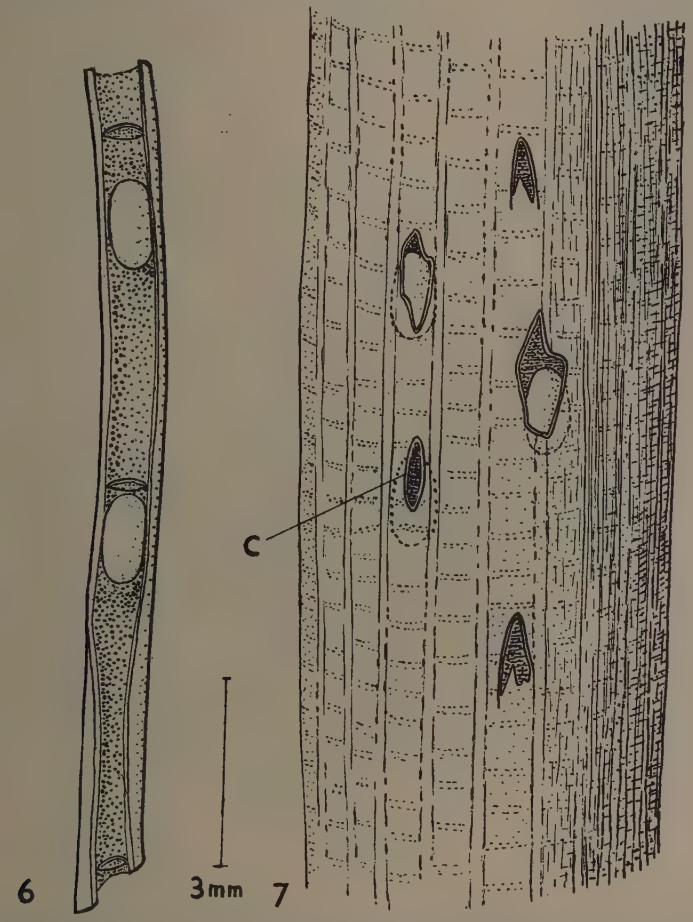
A full investigation of the female reproductive organs of *Ilybius* by a study of serial sections would be of much interest, but has been beyond the scope of the present work. The development of the female genital ducts has been studied in detail by Heberdey in *Hydroporus*, who found that the two genital openings arise as a secondary division of the lumen of the already amalgamated " Anlage " of the eighth and ninth segments, the single lumen becoming separated by the formation of a side wall up to the orifice. The occurrence of two genital openings is well known in various insects of the Panorpoidea (Panorpoid complex), especially in the higher Lepidoptera, and, in the latter order, Heberdey states that the two sexual openings develop from " Anlage " of different segments.

It would be of interest to know how widely spread the two genital openings are amongst the Hydradephaga. I have found that this condition occurs also in *Agabus bipustulatus*, but in this species pressure upon the abdomen does not result in the protrusion of a tubular vagina as in *Ilybius;* instead a trilobed bulging membrane becomes everted dorsally from the vulval lobe. This membrane is normally folded within the vulval lobe behind the ovipore. The vulval lobe forms the floor of the opening to the bursa, so that in this species the two openings are in close proximity. The female reproductive organs in *Agabus* differ strikingly from those of *Ilybius* in that the spermatheca arises from the vagina just below the junction of the paired oviducts, and the bursa is very large and devoid of glands. Stein (1847 : 119–120), who describes the arrangement of these parts in *Colymbetes fuscus* L., states that in *Agabus bipustulatus* they are built upon the same plan. The position of the spermatheca in *A. bipustulatus* is certainly very similar to that shown for *Colymbetes fuscus* by Stein (1847, Plate II, fig. V), but he terms the bursa copulatrix " Untere Hälfte der Scheide ". In *Agabus bipustulatus* (as in *Colymbetes*) the spermatozoa lodged in the bursa copulatrix within a spermatophore during mating must later make their way to the spermatheca. I have found by dissection that, though the vagina and the bursa in *Agabus* have separate openings, the dorsal part of the vagina, to which the lower part of the spermatheca appears to be closely applied, is connected posteriorly just before the base of the vulval lobe with the ventral surface of the the bursa. In some specimens a duct has been traced in this connecting tissue leading from the lower part of the bursa upwards towards the spermatheca. I have no doubt that this is the " Samengang " of Stein (so well shown in his figure of *Colymbetes*), the passage by which the spermatozoa reach the spermatheca. In *Agabus bipustulatus* the spermatheca is curved like a hook and the apical portion forms the " reservoir " of spermatozoa. The fertilization canal arises from the base of the apical zone and extends to the vagina, forming the channel by which the spermatozoa will eventually reach the eggs as they descend from the ovaries. Blunck (1913) has figured a coiled receptaculum seminis in *A. abbreviatus* F. (*undulatus* Schr.) in the same position as I have observed it in *A. bipustulatus*, but of a different shape. Blunck does not, however, record the presence of two genital openings in *A. abbreviatus*.

4. *Plants used for Oviposition*

I have found the eggs of *I. fuliginosus* laid very commonly in the outer leaf stalks of *Alisma plantago-aquatica* L. submerged in pond water, and the beetles in captivity laid readily on this plant. The eggs occur in the sheathing base of the leaf stalk and their presence can be recognized by a gash in the plant tissue, often triangular, sometimes elongate-oval, and frequently a small tongue of leaf tissue projects

over the hole (fig. 7). The egg is sometimes partly visible but it is usually hidden from view, being deeply embedded in the ground tissue of the leaf where it is surrounded by air spaces. Elongated slits are often found with no eggs beneath them, and, as will be described later, a female has often been seen to pierce the plant tissue with her ovipositor without depositing an egg.



FIGS. 6–7—*Ilybius fuliginosus:* (6) two eggs within cylindrical leaf of *Juncus bulbosus* (a strip has been torn from the leaf to expose the eggs); (7) eggs in leaf stalk of *Alisma plantago-aquatica* L. (under two of the slits part of an egg is visible).

In the laboratory, *Juncus articulatus* L. was the plant mostly used for obtaining the eggs, as it could be grown in water under conditions suitable for rearing *Caraphractus* (Jackson, 1958*b*). The eggs were laid within the hollow cylindrical air-filled leaves of this rush and a triangular tongue of leaf tissue was left projecting over the hole. The tongue may face up or down the leaf according to the position the beetle selects for laying. The eggs are mostly laid in the narrow part of the leaves, about 2·9 mm. in diameter, but they may occur in leaves over 4 mm. wide. Occasionally an egg may be laid through the sheathing base of the rush leaf so as to lie between the leaf sheath and the shoot. In such cases the scar is to be found in the leaf sheath, whereas in those species of *Agabus* which lay their eggs under sheathing leaves there

is no scar, and the ovipositor is slipped in at the edge of the leaf (Jackson, 1958*a*).

I have found eggs believed to be those of *fuliginosus* common in the still finer leaves of *Juncus bulbosus* L. (fig. 6) in a quarry pond near St. Andrews, where there was little other vegetation. The eggs occurred principally in the older leaves which were slightly discoloured and covered with algae. Dr. H. Blackler, of St. Andrews University, has kindly determined this alga as a species of *Oedogonium*, and she informs me that its presence on the leaves would increase the oxygen content of the surrounding water. The eggs occurred within leaves varying from 0·9 to 1·45 mm. in diameter, and narrower leaves had scars but no eggs. Most of these eggs were parasitised by *Caraphractus cinctus*, but from five of them *fuliginosus* larvae hatched.

In the leaves of *Juncus* the eggs are usually spaced at intervals and lie parallel to the axis of the leaf. By holding such a leaf to the light it is seen to be riddled with elongated holes, and by ripping up the leaf the eggs are disclosed. Only a very little transparent colourless cement holds them in position, so that they readily fall out on opening the leaf. The orientation of the eggs within the *Juncus* leaves is very precise. In all the eggs I have examined in which incubation was advanced, the ventral surface, where the head with its egg bursters lies, faces the slit in the leaf, the curved dorsal part of the egg projecting into the hollow of the leaf, and the anterior end of the egg is directed towards the opening. When the larva hatches it will have an open " window ", the ovipositor hole, by which to escape. I have occasionally found first instar larvae, fully coloured, imprisoned within a hollow *Juncus* leaf and dead. They must have missed the ovipositor holes and failed to emerge. This orientation of the egg does not wholly correspond to the position of the female in laying, as the dorsal side is not in line with the dorsal side of the mother (Hallez's law, 1886), but this point will be discussed in describing oviposition (p. 47).

I have had eggs laid by *I. fuliginosus* in the stems of water lily leaves, but such stems, when cut off, quickly decay and the eggs may become unhealthy. Wesenberg-Lund (1912 : 20) records that *I. fenestratus* F. lays in stalks of *Potamogeton*. Balfour-Browne (1950 : 161) finds that *I. subaeneus* Er. lays its eggs both inside the stems of water plants and in masses of filamentous algae in an aquarium, and he refers to Bertrand finding the eggs of *I. fuliginosus*, *I. ater* and *I. obscurus* Marsh. in the midst of filamentous algae. I have found that, in the absence of plants with suitable leaves or stalks, *I. fuliginosus* will lay on sphagnum moss, hiding the eggs in the basal leaves of a spikelet or tucking them between the leaves of a bud.

5. *Oviposition*

While I have had females of *fuliginosus* laying in captivity each summer since 1955, it was only last year (1958) that I observed oviposition. After various unsuccessful attempts to view the process adequately with the beetles confined in glass jars, I found that by placing a laying female in a large glass tube (tubes 4 inches by 1¼ inches were used) with leaves of *Juncus articulatus*, and inserting the cork, it was possible to get an excellent view of oviposition, and even to observe the process under the dissecting microscope. By leaving the females overnight in the tubes (covered with muslin instead of cork) and with no plants, they were usually ready to lay soon after the *Juncus* was introduced.

Before laying, a female may remain for some time standing on a leaf with the tip of the abdomen projecting, so that part of the genital segment is exposed and the ovipositor blades protrude parallel to, but above, the leaf. This attitude does not indicate that oviposition is imminent, but is probably caused by the pressure of the ripe eggs in the abdomen. When about to lay the female takes up a stance on the *Juncus* leaf, facing either up or down, and the first sign of the commencement of oviposition is the enlargement of the bubble of air at the apex of the abdomen owing

to the further protrusion of the genital segment. While the first cut in the leaf is made, the seventh sternum is held slanting downwards so that its outer edge appears to scrape the leaf, whilst the extreme apex of the ovipositor blades, which are awl-like and free of teeth, bore into the leaf at right angles to the surface. The two blades are supported for this initial boring action by the seventh sternite, for they fit into the wide emargination already described in the apex of that sclerite. This emargination allows the ovipositor blades to pierce the plant tissue whilst being held almost perpendicular to the leaf surface. No such emargination occurs in the last visible sternite of *Agabus bipustulatus* L., which has no piercing ovipositor and lays its eggs in a different way (Jackson, 1958*a*).

After the initial downward cut into the leaf tissue is made by the *Ilybius* female, the apex of the abdomen straightens and the two ovipositor blades commence a rhythmic saw-like action. The most obvious evidence of this is the quick jerky movement of the air bubble, now greatly enlarged (fig. 5), which may be seen to move backwards and forwards as the apex of the abdomen is protruded and withdrawn during the sawing motion of the ovipositor blades. The blades are pushed out and partly withdrawn again and again at high speed, with a vigorous rhythmic movement, and the tongue of leaf tissue can be seen rising up between the blades as the cuts extend, for the blades diverge distally so as to cut a widening gash. During the cutting process a rasping noise is audible. At this stage the triangular eighth sternites, though fully extended, play no part; they project horizontally above and parallel to the leaf. When the cut is completed there is a pause, and the creamy-white egg is deposited in the hole below the ovipositor blades. At this moment the eighth tergum, the pygidium, is lowered, and the eighth sternites, hitherto inactive, are curved down to touch the leaf above the hole with a sort of quick patting action, as though to flatten down the tongue of projecting leaf tissue. The operation is now completed and the beetle moves off to lay at a fresh spot or to rise to the surface to renew its air supply. What actually happens during the patting action of eighth sternites is unknown. It would appear probable that, at some stage in the laying, the egg is turned round so that its dorsal surface—which should be in line with the dorsum of the female—is placed ventrally (see p. 46). The whole process of oviposition (which has been observed on several occasions) is accomplished at great speed, occupying from one minute five seconds to three minutes.

The females usually face down the leaf when laying so that the tongue of cut leaf projects downwards. Sometimes the beetles may make a scar of the usual type without laying, or they may merely produce an elongated cut without sawing the tongue. No egg has been found under such a gash in a leaf of *Juncus articulatus*, but in an *Alisma* leaf stalk, in which the epidermis is probably softer to cut, an egg may be embedded without a tongue of leaf projecting (fig. 7 *C*).

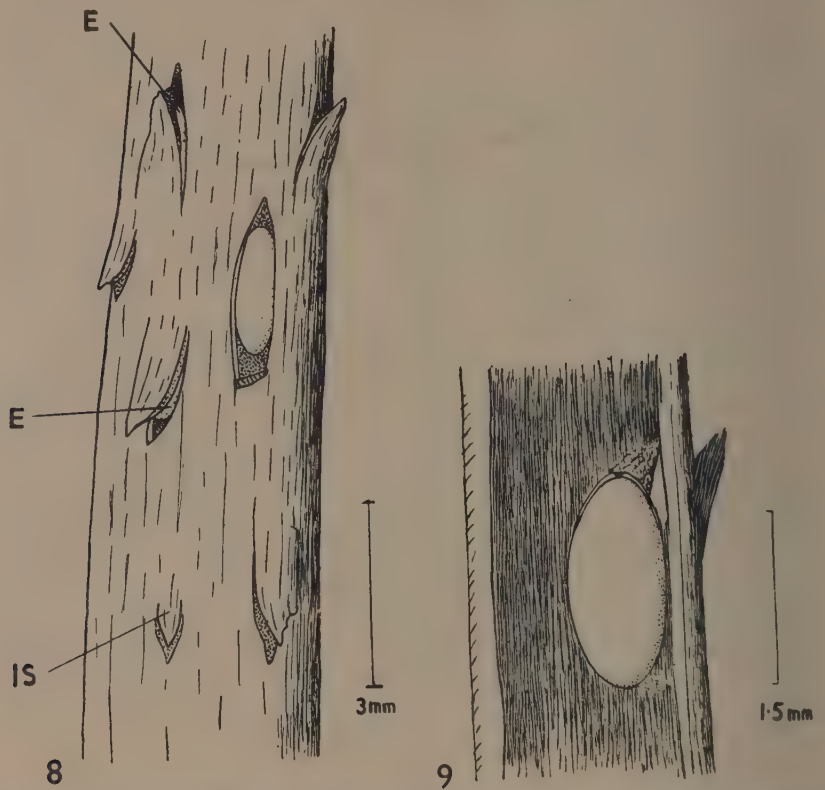
6. *The Egg*

The egg of *I. fuliginosus* is creamy-white, elongate-oval with the dorsal surface slightly concave and the ventral side nearly straight. It is affixed to the plant by a little transparent gelatinous cement, which later darkens. The chorion is very distinct as a translucent membrane surrounding the egg, and the micropylar area forms a white spot at the anterior end. Eggs within 24 hours of laying measure from 1·24 by 0·59 to 1·33 by 0·50 mm. They increase in size to 1·43 by 0·8 to 1·53 by 0·76. The chorion persists and may be seen in the hatched egg.

7. *Hatching*

When the larva is ready to emerge it lies in a curve within the egg, the mandibles occupying a position practically in the centre of the ventral surface. The egg-bursters on the frons are peg-like and each is situated at the anterior apex of a triangular pigmented area which is much larger than the round pigmented spot around the egg-

bursters in the larva of *Agabus bipustulatus*. In an egg eight to nine days old, a cellular membrane, presumably the serosa, was seen to surround the entire embryo, and the pleuropodia on the first abdominal segment were distinct. The next day the embryonic cuticle was visible surrounding the embryo and its various appendages, as in *A. bipustulatus* (Jackson, 1957). Hatching occurs in from 16 to 18 days after laying when the eggs are kept in an unheated room. An egg with fully developed larva mounted in water was seen to hatch by the larva pressing its head forwards and then slithering out through a median slit which extended half way down the egg from the anterior pole. It brought out the delicate embryonic cuticle with it, but left it sticking to the egg.



FIGS. 8–9.—*Ilybius ater* Degeer: (8) egg scars made in leaf of *Juncus articulatus* (the tongue of leaf tissue over one egg has been cut away to expose egg); (9) egg within a leaf of *J. articulatus* from which a strip has been cut away (the transparent cement attaching egg to leaf is indicated at both ends of the egg).

III. *Ilybius ater* Degeer

Since the laying habits of this species are so similar to those of *I. fuliginosus* a detailed account is unnecessary.

I. ater is much less common in Fife than *I. fuliginosus*. I have taken it only occasionally in ponds and lochs from the end of April until the end of July, and a female in captivity lived from early July to January. Oviposition occurred from early June till the latter part of August. It is a much larger beetle than *fuliginosus*, so that its eggs, and the scars in the leaf tissue, are easily recognized.

The female genitalia are like those of *fuliginosus*, but the ovipositor blades bear

more teeth, from 14 to 16, and the median sclerotic strip at the base of the vulval lobe (fig. 4, *MSS.*) is triangular instead of linear. On pressing the abdomen of a newly killed beetle the vagina may be forced out of the vulval lobe as a longish tube just as in *I. fuliginosus*. Eggs have been found in leaf stalks of *Alisma* growing in a pond and they often occur close together. In captivity the females have laid in leaves of *Juncus articulatus* (fig. 8) and *J. communis* Mey. and in leaf stalks of water lilies. Eggs may be laid amongst sphagnum in the absence of these other plants.

My observations on egg-laying are limited to two females, both from Fife. The first, collected in early July 1957, must have been unmated as none of her eggs was fertile. After cutting a hole with her ovipositor in the leaf tissue she very often deposited the egg on the surface and sometimes would at once turn round and eat the egg. In one instance she tore part of a water lily leaf into shreds in order to get at an egg she had deposited in it. The second female was collected in early May 1958, at a time when I had a living male to place with her. She subsequently laid numbers of fertile eggs, depositing them in the holes she had cut in the *Juncus* (fig. 8), and only rarely leaving an egg on the surface of a leaf. Of five eggs not inserted only one later showed an embryo ; the others decayed.

The egg (fig. 9) is rather differently shaped from that of *I. fuliginosus*, being wider in the centre and not so flattened on the ventral side. A newly laid egg measured 1·92 by 0·80 mm. ; other eggs, measured five to six days after laying, varied from 1·85 by 0·90 to 1·96 by 0·97 mm. The egg is fastened in the leaf with a little cement.

The larva hatches by means of a small longitudinal rent on the ventral surface of the egg near the anterior end and facing the hole in the leaf made by the ovipositor. The incubation period lasts about a month. The larva, when ready to emerge, shows two conspicuous triangular areas on the frons, each of which bears the egg-burster ta its anterior apex. These triangular areas are dark in contrast to the rest of the head, but once the larva emerges they are not so noticeable, as the rest of the cuticle then becomes dark. From eggs laid during the late summer I had larvae in the third instar in November, but during January they died, though not full grown.

IV. Discussion

In the genus *Ilybius* the ovipositor blades with their saw-like teeth are remarkably well adapted for the insertion of the eggs within the living tissues of aquatic plants. The laying apparatus is a complicated structure involving the coaptation of many sclerites. The principal levers for the piercing action of the ovipositor are the strongly sclerotic valvifers, which serve for the attachment of powerful muscles. This piercing type of ovipositor is correlated with a modification of the seventh sternum in the form of a central emargination, which is a characteristic feature of female *Ilybius*. The ovipositor in this genus is more highly developed than the simpler type found in *Agabus*, in which the eggs are usually laid externally on the leaves, or slipped under the sheathing bases of the leaves of various aquatic plants.

The eggs of the two species of *Ilybius* studied are inserted within the hollow air-filled leaves of *Juncus* or amongst the air spaces in the *Alisma* leaf stalk. In this position, surrounded with air and moisture, the eggs must have the best chance of survival. They are so completely hidden that they must escape the attack of many predators. In healthy plant tissues they will be immune from attacks of micro-organisms which are so prone to develop on water beetle eggs if the plants decay. Only a few of the eggs, believed to be those of *I. fuliginosus*, found within slightly discoloured leaves of *Juncus bulbosus* succumbed to micro-organisms, and the presence of algae on such leaves would increase the oxygen content of the surrounding water (p. 46) and help to keep the eggs healthy.

In view of this highly specialized method of oviposition it is interesting to find that, in the absence of suitable plants, the female can abandon the cutting operation and deposit the eggs on sphagnum, or, as recorded by others, on filamentous algae. It shows a versatility which is surprising and will no doubt result in the survival of some at least of her progeny in waters, perhaps of recent formation, where other aquatic vegetation has not become established. The eggs deposited on the sphagnum by *I. fuliginosus* were hidden in the basal leaves of a spikelet or carefully tucked between the leaves of a bud, and such eggs duly hatched. I have some reason to doubt whether the eggs which a female fails to place in a hole she has cut and which drop to the bottom, survive. I believe these " misplaced " eggs are similar to the dropped eggs of *Dytiscus marginalis* L., which I find never develop and which Joly (1945) suggested were virgin.[1] He considered that fertilization was a reflex, having for its starting point the efforts of the ovipositor to push the egg into the stalk of a plant. It is clear that with *I. fuliginosus*, although there is no cutting action of the ovipositor when eggs are laid on sphagnum, the careful placing of these eggs amongst the leaves must result in a normal release of spermatozoa during laying. It is evident that the eggs of *Ilybius* can develop either surrounded with air within tissues of plants or in direct contact with water, as when laid on sphagnum. It is interesting to find that such comparatively large beetles can insert their eggs with precision in the very slender floating leaves of *Juncus*, so much narrower than themselves. Eggs of *I. ater* have been laid in leaves of *Juncus articulatus* only 1·7 mm. in diameter, while eggs, believed to be of *fuliginosus*, have been found in leaves of *J. bulbosus* only 0·9 mm. wide (p. 46).

The method of laying within plant tissues affords no protection against the Mymarid, *Caraphractus cinctus*, as the females have no difficulty in parasitising the eggs laid in *Juncus* and *Alisma*, sometimes inserting the ovipositor through the " tongue " of the leaf scar into the egg beneath, or else laying directly into the egg at the side of the tongue. Eggs of both *I. fuliginosus* and *I. ater* were heavily parasitised by this Mymarid in my experiments, and probably any plant soft enough for the ovipositor of the beetle to cut will also be suitable for the female *Caraphractus* to pierce with her ovipositor.

V. Summary

The female genitalia in *Ilybius fuliginosus* F. are described. The valvifer is strongly developed and the ovipositor blades bear saw-like teeth. The genitalia of *I. ater* Deg. are closely similar.

Two genital openings are present in *Ilybius*, the upper leading into the bursa copulatrix and the lower into the vagina at the vulval lobe. Two genital orifices have also been observed in *Agabus bipustulatus* L.

An account is given of the position of the eggs of *Ilybius fuliginosus* F. in the tissues of various aquatic plants. In the cylindrical leaves of *Juncus* they are placed within the air filled hollow of the leaf, and in *Alisma plantago-aquatica* amongst the air spaces in the plant tissue.

Oviposition in the leaves of *Juncus articulatus* by *I. fuliginosus* is described. The initial incision is made by the two ovipositor blades while they are supported within the apical emargination of the seventh sternum. This sternum is held downwards, touching the leaf and the tips of the blades penetrate the epidermis at right angles to the leaf. The apex of the abdomen then straightens and the two blades commence a rhythmic saw-like action whereby a tongue of the plant tissue is cut, and the egg deposited in the hole. *I. ater* Deg. lays her eggs similarly.

[1] A female of *D. semisulcatus* Müll., in the course of laying many fertile eggs inserted in *Juncus* shoots, left one free on the stalk and this egg duly hatched.

In the absence of plants with suitable leaves or stalks, the female *Ilybius* will lay on sphagnum, and the eggs, though surrounded with water instead of air-filled plant tissues, duly hatch.

The eggs of *I. fuliginosus* and *I. ater* are described. The egg is orientated within the rush leaf in such a position that the larva faces the oviposition hole and so usually escapes into the water.

VI. Explanation of Lettering

A, anus.
AO, apparent orifice of vulval lobe, where vaginal membrane is inflexed.
BO, base of ovipositor blade.
C, elongated cut in leaf below which an egg has been inserted.
E, egg.
IM, intersegmental membrane.
IS, incompleted scar with no egg laid beneath it.
M, membrane.
MSS, median sclerotic strip at base of vulval lobe.
OB, ovipositor blade.
Os, ostium of bursa.
Ov, ovipore, concealed under fold of vaginal membrane.
Pl, pleurite.
Plm, pleural membrane.
Sr, side border (" Seitenrand ") of seventh sternite (Korschelt, 1923 : 73).
V, valvifer.
Va, vagina.
VL, vulval lobe.
VP, ventral surface of pygidium (eighth tergum).
VS, vulval sclerite.
X, position of aperture of anal gland reservoir.
Y, area of valvifer lying near anus.
Z, probable position of vulval lobe within leaf during laying.
7, seventh sternum.
8, eighth sternite.
VIII, eighth tergum (pygidium).

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